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Impact of tumour histological subtype on chemotherapy outcome in advanced oesophageal cancer

Running title: Oesophageal cancer histology and chemotherapy response

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ABSTRACT

Aim: To investigate the impact of histology on outcome in advanced oesophageal cancer treated with first-line fluoropyrimidine-based chemotherapy.

Patients and Methods: Individual patient data were pooled from three randomised phase III trials of fluoropyrimidine-based chemotherapy +/- platinum/anthracycline in patients with advanced, untreated gastroesophageal adenocarcinoma or squamous cell carcinoma (SCC) randomised between 1994 and 2005. The primary endpoint was overall survival of oesophageal cancer patients according to histology. Secondary endpoints were response rates and a toxicity composite endpoint.

Results: Of the total 1836 randomised patients, 973 patients (53%) were eligible (707 patients with gastric cancer were excluded), 841 (86%) had adenocarcinoma and 132 (14%) had SCC. There was no significant difference in survival between patients with adenocarcinoma and SCC, with median overall survivals of 9.5 versus 7.6 months (HR 0.85, 95% CI 0.70-1.03, $p=0.09$) and one-year survivals of 38.8% versus 28.2% respectively. The overall response rate to chemotherapy was 44% for adenocarcinoma versus 33% for SCC ($p=0.01$). There was no difference in the frequency of the toxicity composite endpoint between the two groups.

Conclusions: There was no significant difference in survival between adenocarcinoma and SCC in patients with advanced oesophageal cancer treated with fluoropyrimidine-based chemotherapy despite a trend for worse survival and less chemo-sensitivity in SCC. Tolerance to treatment was similar in both groups. This analysis highlights the unmet need for SCC-specific studies in advanced oesophageal cancer and will aid in the design of future trials of targeted agents.

CORE TIP:

There is a lack of published data on differential treatment response according to histology in oesophageal cancer. This paper shows improved response rates with first-line chemotherapy and a trend towards improved survival in adenocarcinoma compared to SCC. It is increasingly recognised that these histological subtypes represent discrete disease entities with divergent treatment pathways in both the early stage and advanced settings. Novel treatments in SCC remain sparse and there are few dedicated trials in this subtype. This data highlights the poor outcomes seen with chemotherapy alone and the need for further research, particularly for SCC.

INTRODUCTION

Oesophageal cancer is the eighth most common malignancy worldwide with an estimated 456 000 new cases and 400 000 deaths worldwide in 2012, ranking it the sixth most common cause of cancer-related deaths^[1]. Despite recent advances in genetic and molecular characterisation and the development of novel targeted agents survival rates for oesophageal carcinoma have changed little for many decades, and outcomes for advanced disease remain poor. Worldwide, squamous cell carcinoma (SCC) is the predominant histological subtype however in North America and Northern Europe the incidence of oesophageal adenocarcinoma has increased in the last 20 years whereas that of SCC has decreased^[2, 3]. This is likely to reflect the distinct aetiological factors implicated in the development of the two diseases. SCC is strongly correlated with excessive alcohol consumption, cigarette smoking and poor socioeconomic status whereas adenocarcinoma is associated with obesity and gastroesophageal reflux disease (GORD)^[3-6]. Thus the rise in adenocarcinoma may be in part due to changing lifestyle factors in Western populations^[3]. Genomic technology has been applied to both gastric and oesophageal cancer in an effort to improve understanding and stratification on a genetic and molecular level, with emerging differences in the genetic landscape between the two histological subtypes suggesting a need for more tailored therapeutic strategies^[7, 8]. Historically however treatment patterns for both subtypes have been similar, with many clinical trials evaluating chemotherapy conducted since the mid-1990s including patients with gastric, oesophageal, or oesophagogastric junction (OGJ) cancer, regardless of histology. Similarly, studies in early stage oesophageal cancer often include both histological subtypes, such as the recent CROSS trial evaluating neoadjuvant chemo-radiation. This identified disparities in outcome according to histology, with a statistically significant overall survival benefit seen only in the smaller SCC cohort^[9].

In the advanced disease setting cisplatin/ fluorouracil with the possible addition of a third drug- either epirubicin or a taxane- is commonly used as a first-line chemotherapy, and second-line agents include irinotecan, docetaxel and paclitaxel^{[10] [11]}. More recently treatment patterns have diverged, with the introduction of novel molecularly-targeted therapy for gastroesophageal adenocarcinomas. Notably effective therapies targeting *HER2* (trastuzumab) and the vascular endothelial growth factor receptor 2 (ramucirumab) are applicable only to adenocarcinomas^[12-14].

Three randomised phase III studies of fluoropyrimidine-based combination chemotherapy have been published in patients with advanced gastroesophageal cancer including oesophageal SCC and adenocarcinoma^{[10],[15],[16]}. In multivariate Cox regression analysis histology was not identified as a variable impacting on survival, however patients with SCC accounted for less than 10% of the patients in each trial. Although SCC normally represent a small minority of patients enrolled on most clinical trials it is not clear what influence histologic subtype exerts on response rate or survival duration in patients treated with cytotoxic chemotherapy regimens for metastatic disease, and SCC has been associated with both worse, better or similar outcomes to adenocarcinoma^[17-19]. The distinct epidemiological, genetic and molecular characteristics of SCC as compared to adenocarcinoma could potentially influence response to therapies administered in the advanced disease setting. In this pooled analysis of the three randomised phase III studies which included patients with both advanced oesophageal SCC and adenocarcinoma, we aimed to evaluate whether there was a differential treatment effect according to histology.

PATIENTS and METHODS

Patients and Treatments

Between 1994 and 2005, 1836 patients were randomised predominantly from the UK in three multi-centre randomised controlled trials of fluoropyrimidine-based chemotherapy in patients with untreated locally advanced or metastatic carcinoma of oesophagus, OGJ, or stomach^[10, 15, 16]. Similar eligibility criteria were applied in the three trials; patients had histologically confirmed inoperable adenocarcinoma, squamous cell carcinoma or undifferentiated carcinoma of the oesophagus, OGJ or stomach, adequate haematological, renal and hepatic function and an Eastern Co-operative Oncology Group performance status (PS) of 0-2. Written informed consent was obtained from all patients and all three studies were approved by the Scientific and Research Ethics Committees of the participating institutions.

The first study randomised 580 patients between 1995 and 1998 to treatment with ECF (epirubicin 50 mg/m² intravenously [IV] and cisplatin 60 mg/m² IV infusion with hydration on day 1 plus 5-FU 200 mg/m²/day by protracted venous infusion [PVI]) or MCF (mitomycin C [MMC] 7 mg/m² on day 1 every six weeks, cisplatin 50 mg/m² IV day 1 and PVI-5-FU 300 mg/m²/day)^[15]. The second study randomised 254 patients between 1994 and 2001 to PVI 5-FU (300 mg/m²/day) or the same dose of PVI 5-FU plus MMC (7 mg/m² every six weeks)^[16]. The third study conducted randomised 1,002 patients between 2000 and 2005 to ECF, ECX (X denotes capecitabine given at a dose of 625 mg/m² twice a day continuously), EOF (O denotes oxaliplatin 130 mg/m² on day 1 every three weeks replacing cisplatin) and EOX^[10].

A maximum of 8 cycles of chemotherapy (24 weeks) with response assessment computed tomography (CT) scans at 12 and 24 weeks was stipulated in the three study protocols. Overall survival (OS) was the primary outcome measure in these trials and toxicity data was recorded at each treatment visit every three weeks.

Only eligible patients with squamous carcinoma or oesophageal/oesophagogastric junctional (OGJ) adenocarcinoma who received at least one dose of chemotherapy were included in this analysis which was based on individual patient data from these trials.

Statistical Analysis

OS was the primary endpoint of this pooled analysis and was calculated from the date of randomisation until death from any cause, or censored at the date of last follow-up for surviving patients according to the Kaplan-Meier method. Survival analyses were performed on the eligible population and compared between patients with SCC and adenocarcinoma using the log rank test. Multivariate survival analysis was performed using Cox proportional hazard model and stratified for treatment centres. The following factors were included: histology, gender, primary site (oesophagus vs. OGJ), liver or peritoneal metastases (presence vs. absence), serum alkaline phosphatase (<100 vs. ≥ 100 U/l) and performance status (0-1 vs. 2) based on previously-identified prognostic factors in advanced OG cancer^[20, 21], as well as treatment arm and trial.

Objective response rates between SCC and adenocarcinoma were compared using Chi Square test. A chemotherapy-specific toxicity composite endpoint (TCE) was constructed as a surrogate for undesirable cytotoxic-related toxicities. TCE was defined as the first occurrence of grade 3 or 4 diarrhoea, neutropenia, febrile neutropenia, fever, infection, nausea & vomiting or grade ≥ 2 renal or neurotoxicity. TCE was compared between the two histological subtypes using Chi Square test. Time to TCE was compared between SCC and adenocarcinoma using log rank test.

Two-sided p value of less than 0.05 were considered significant for the overall survival endpoint, and 95% confidence intervals (CI) quoted. Analyses were performed using SPSS package version 23 (SPSS Inc, Chicago IL).

RESULTS

Patient Characteristics

Of the 1836 patients randomised to the three trials, 973 patients (53%) were eligible for this pooled analysis as indicated in Figure 1. 707 of the 1836 patients (39%) were excluded due to the primary tumour origin being gastric. Of the 973 eligible patients 841 (86%) had adenocarcinoma and 132 (14%) had SCC. Baseline patient characteristics are shown in Table 1. These were broadly balanced between the two histological sub-types except that predictably a greater proportion of adenocarcinoma occurred at the OGJ with metastases to the liver/peritoneum, and there were more males with adenocarcinoma.

Survival

At the time of the data cut-off, 863 of the 973 patients (88%) had died and the median follow-up for surviving patients was 19 months. The median survival for the whole cohort of 973 eligible patients was 9.4 months (95% CI 8.82-9.99). One year survival was 37.3% (95% CI 37.27-37.33) and 2 year survival was 13.5% (95% CI 13.48 - 13.52). There was no significant difference in survival between patients with adenocarcinoma and SCC, with median OS of 9.5 versus 7.6 months (HR 0.85, 95% CI 0.70-1.03, $p=0.09$), although the curves did appear to separate between 6 months to 2 years suggestive of a poorer survival for SCC during this period (Figure 2A). One and two year survival figures for adenocarcinoma were 38.8% (95% CI 38.77-38.83) and 13.6% (95% CI 13.57-13.63) respectively and for SCC were 28.2% (95% CI 28.12-28.28) and 12.3% (95% CI 12.24-12.36). When considering only 'true' oesophageal cancer patients only and excluding those with junctional tumours, there was again no significant difference in survival between patients with adenocarcinoma ($n=438$) and SCC ($n=117$), with median OS of 9.5 versus 7.7 months (HR 0.91,

95% CI 0.73-1.13, $p=0.38$) (Figure 2B). In multivariate analysis, previously identified known prognostic factors of performance status, liver/peritoneal metastases and alkaline phosphatase were all significant. Histology and site of primary tumour were not shown to be significant prognostic factors. For effect of treatment received there was a significant association of treatment within trial 2¹⁶- which did not incorporate a platinum component into either treatment arm- with poorer outcome (Table 2).

Response and Toxicity

The objective response rate to chemotherapy (Table 3) was significantly higher for patients with adenocarcinoma compared to SCC (44% vs. 33%, $p=0.01$). A greater proportion of patients with SCC compared to adenocarcinoma progressed during chemotherapy (29% vs. 19%, $p=0.01$) and the proportion of patients with stable disease was similar for both histological subtypes. There was no difference in the proportion of patients experiencing the toxicity composite endpoint (TCE) for adenocarcinoma as compared to SCC (45% versus 44%, $p=0.77$) (Table 3). Similarly there was no difference in the time to development of TCE (Figure 3) between the histological subtypes (HR 0.98, 95% CI 0.74–1.29, $p=0.9$).

DISCUSSION

This study represents the largest pooled analysis of differential chemotherapy effects in patients with advanced oesophageal adenocarcinoma and SCC undergoing fluoropyrimidine-based chemotherapy in randomised phase III controlled trials with mature survival data. All three analysed trials incorporated a fluoropyrimidine in each treatment arm, and two of the trials included a platinum agent in each arm. In this pooled analysis there was no significant difference in overall survival between patients with adenocarcinoma compared to those with SCC with median overall survivals of 9.5 versus 7.6 months (HR 0.85, $p=0.09$) respectively. A possible limitation of interpretation of this data is the imbalanced distribution of histological

subtype between oesophageal and OGJ cancers. As expected, the proportion of SCC histology was higher in the oesophageal only group as compared to the total cohort of oesophageal and OGJ patients (21% versus 14%). A further analysis excluding OGJ patients however also did not show a significant difference in median OS between adenocarcinoma and SCC (9.5 versus 7.7 months, HR 0.91, $p=0.38$). Histology and site of primary tumour were not shown to be predictors of survival in multivariate analysis, consistent with previously reported prognostic variables in oesophageal and gastric cancer based on smaller analyses^[20, 22, 23]. The survival curves did appear to separate between 6 months and 2 years, with SCC patients appearing to have worse survival during this period, but the curves then overlapped from two years onwards. Lack of a statistically significant difference in survival in the presence of a trend could reflect that this pooled analysis remains underpowered. Although the potential for heterogeneity may confound interpretation of data from pooled analyses, the eligibility criteria for these three trials were similar, individual patient data were used to strengthen the analysis, treatment arms and trials were incorporated in the multivariate analysis and survival outcomes from ECF, evaluated in the two largest trials^[10, 15], were consistent. Inclusion of patients with advanced SCC in these studies was controversial in terms of potentially creating a heterogeneous study population however based on the current analysis survival outcomes with standard chemotherapy are not significantly different with SCC compared to adenocarcinoma, although there may be a trend towards worse survival. The only differential treatment effect noted was a significant difference in objective response rates between adenocarcinoma and SCC (44% vs. 33% respectively). A greater proportion of SCC patients also progressed during treatment (29% vs. 19%), suggesting that oesophageal SCC may be less chemo-sensitive than adenocarcinoma.

There was no difference in time to development of TCE or of the proportion of patients with TCE between the two histological sub-types. A difference

might have been expected given the association of co-morbid conditions with SCC. However, within clinical trials there may be selection bias favouring inclusion of fitter patients (patients with a performance status of 2 comprised only 15% of the pooled patient population in this analysis). Although this does potentially limit extrapolation of the results of this analysis to patients with SCC in the general population this would apply to most randomised controlled trials in this disease.

Application of genomic technology is revealing increasing differences between the histological subtypes of oesophageal cancer on a genetic and molecular level. In an analysis performed by the Cancer Genome Atlas, four gastric cancer subtypes have been proposed: tumours positive for Epstein-Barr virus (EBV), microsatellite unstable tumours (MSI), genomically stable (GS) tumours and tumours with chromosomal instability (CIN)^[24]. Each subtype was found throughout the stomach, but CIN tumours showed elevated frequency in the OGJ and cardia. In CIN tumours genomic amplifications of receptor tyrosine kinases such as *VEGFA* and cell cycle mediators such as *CCND1* and *CDK6* with potentially relevant clinical implications were found with increased frequency. Specific to oesophageal adenocarcinomas, a sequencing study of 149 tumours by a US group published in Nature Medicine in 2013 confirmed recurrent mutations in known cancer-driving genes including *TP53*, *CDKN2A*, *SMAD4*, *ARID1A* and *PIK3CA*^[25]. Similarly, a number of recent studies have applied NGS to the study of oesophageal SCC, demonstrating recurrent mutations in known oncogenic drivers including *TP53*, *NOTCH1*, *PIK3CA* and *FAT1*, as well as amplifications in *CCND1* and *CDKN2A*^[26]. The cell cycle regulation pathway is one of the most consistently altered in oesophageal SCC, where mutations are observed at a high frequency and are associated with poor prognosis and metastasis^[27] ^[28]. A recent study has compared the genomic profiles of 71 SCC and 231 oesophageal adenocarcinomas, focusing on the identification of therapeutically relevant genomic alterations in both groups^[8]. Similarly high

frequencies of clinically relevant genomic alterations were found in both histological subtypes; however the profiles of genomic alterations in the two diseases differed substantially. *KRAS* and *HER2* were more frequently altered in adenocarcinoma, while *MTOR* pathway genes (*PIK3CA*, *PTEN*) and *NOTCH1* were more frequently altered in SCC. Exploitation of the molecular differences between the two histological sub-types may help direct optimal application of targeted therapies in this disease.

Although our data is historical, the chemotherapy landscape for oesophageal cancers has not changed significantly in the intervening years. Targeted treatments for oesophageal adenocarcinomas are now in routine clinical use and starting to provide tangible improvement to patient outcomes, however there remains a relative lack of both applied research and effective treatments for advanced SCC. Given small patient numbers and apparently declining incidence, further randomised SCC-specific phase III trials of systemic therapy in advanced oesophageal cancer in Western populations will be challenging. Future improvements in outcome are likely to come from smaller studies investigating cohorts of patients enriched for discrete genetic aberrations, or from the use of combination immunotherapeutic approaches. Optimising the design of such studies using appropriate chemotherapies as either comparators or backbones to newer investigative agents requires an understanding of differential effectiveness and toxicity of standard chemotherapy regimes. This analysis demonstrated no significant difference in survival or tolerance to chemotherapy between patients with adenocarcinoma or SCC. Given the poor outcomes seen with chemotherapy it reinforces the need for SCC-specific trials in advanced oesophageal cancer.

COMMENTS

1. Background

The two main histological subtypes of oesophageal cancer, adenocarcinoma and SCC, are increasingly regarded as discrete disease entities with divergent treatment pathways. This is reflected in recent international guidance from both the National Comprehensive Cancer Institute and European Society of Medical Oncology, which recommend differing treatment approaches in early stage and, to a lesser extent, late stage disease dependent on histology^{[29][30]}.

2. Research frontiers

Although the chemotherapy landscape for advanced oesophageal cancer has not changed in recent years, improved understanding of the molecular and genomic underpinnings of the disease have led to tangible improvements in outcome, with effective biological targeted agents such as trastuzumab and ramucirumab making a tangible difference to patient outcomes. The clinical application of such targeted agents has so far however been limited to the adenocarcinoma subtype. Emerging data on the use of immunotherapy suggests that it will also play a role in this condition. Recent preliminary data from trials of immunotherapy agents such as the KEYNOTE 028 study evaluating use of the anti-PD1 agent pembrolizumab in advanced oesophageal cancer have reported promising signal in both adenocarcinoma and SCC patients, and studies of immunotherapy in both histological subtypes are ongoing^[31]. Although SCC remains a significant health problem on a global scale, incidence in Western populations is declining and further large scale randomised trials restricted to this subtype are unlikely.

3. Innovations and breakthroughs

There is a lack of randomised data on differential chemotherapy response according to histology in oesophageal cancer. This paper shows that adenocarcinomas had a significantly higher response rate to first line

fluoropyrimidine-based chemotherapy than SCC. Although there was also a trend towards improved survival outcomes this did not reach statistical significance. This data confirms the generally poor outcomes seen with chemotherapy in advanced oesophageal cancer and suggests that oesophageal SCC may be a less chemotherapy-sensitive disease than adenocarcinoma.

4. Applications

Given the now established role of targeted agents in the management of advanced oesophageal adenocarcinoma and an emerging potential role for immunotherapeutic approaches it is possible that treatment pathways for the two subtypes will further diverge. Improvements in outcome are likely to come from smaller studies investigating targeted agents or combination immunotherapeutic approaches. Optimising the design of such studies using appropriate chemotherapies as either comparators or backbones to newer investigative agents requires knowledge of the differential effectiveness and toxicity of chemotherapy.

Table 1: Patient Characteristics

	Adeno	SCC	Total
Number of Patients	841	132	973
Median age (range)	62 (22-84)	60 (37-77)	61 (22-84)
Gender			
Male	730 (87%)	95 (72%)	825 (85%)
Female	111 (13%)	37 (28%)	148 (15%)
Performance Status*			
0	223 (27%)	33 (25%)	256 (26%)
1	489 (58%)	75 (57%)	564 (58%)
2	127 (15%)	23 (18%)	150 (15%)
Sub-site			
Oesophagus	438 (52%)	117 (89%)	555 (57%)
GOJ	403 (48%)	15 (11%)	418 (43%)
Extent of Disease**			
Locally Advanced	219 (26%)	36 (27%)	255 (26%)
Metastatic	622 (74%)	95 (72%)	717 (74%)
Location of Metastases			
Liver	340 (46%)	46 (35%)	386 (40%)
Peritoneum	41 (5%)	4 (3%)	45 (4.5%)
Lung	136 (16%)	18 (14%)	154 (16%)

* PS unknown or 3 in < 1% of all patients

** Data was unavailable for one patient in the SCC group

Table 2: Multivariate analyses of overall survival

		Univariate analysis			Multivariate analysis		
Factors		HR	95% CI	P value	HR	95% CI	P value
Overall survival							
Histological Arm	Adenocarcinoma (r)	1.000					
	SCC	1.196	0.972 – 1.471	0.089			
Sex	Female (r)	1.000					
	Male	0.983	0.807 – 1.196	0.983			
Subsite	Oesophagus (r)	1.000					
	OGJ	0.988	0.853 – 1.145	0.876			
Liver Mets	No (r)	1.000			1.000		
	Yes	1.671	1.433 – 1.948	<0.001	1.581	1.341 – 1.863	<0.001
Peritoneal Mets	No (r)	1.000			1.000		
	Yes	2.290	1.583 – 3.314	<0.01	2.190	1.503 – 3.191	<0.001
ALP	<100U/I (r)	1.000			1.000		
	≥100U/I	1.608	1.357 – 1.908	<0.001	1.287	1.072 – 1.544	0.007
Performance Score	0 – 1 (r)	1.000			1.000		
	2 - 3	2.140	1.754 – 2.611	<0.001	1.703	1.374 – 2.110	<0.001
Treatment Arm	EOX (r)	1.000					
	EOF	1.122	0.848 – 1.484	0.420			
	ECX	1.139	0.862 – 1.505	0.361			

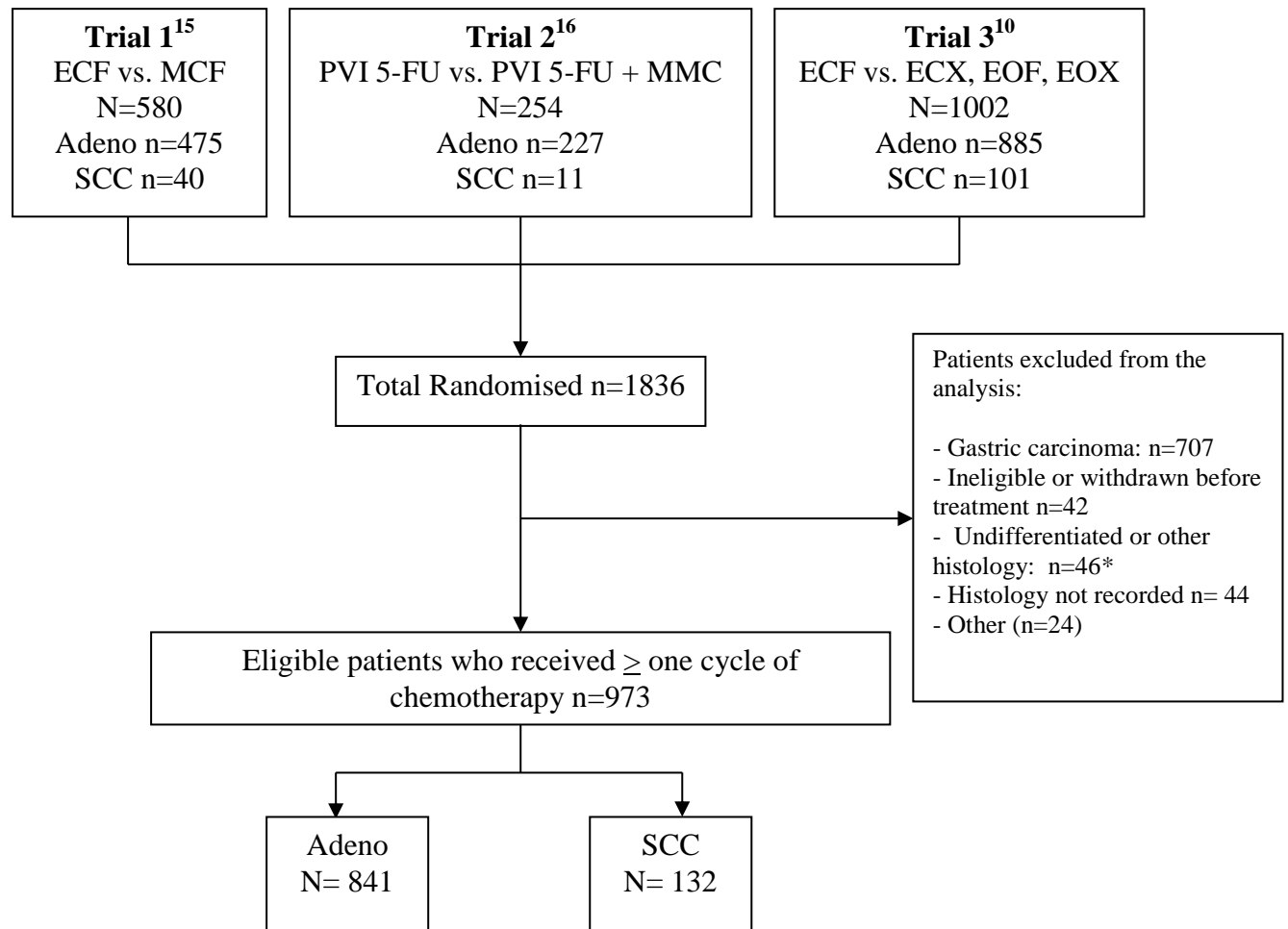
	ECF	1.175	0.916 – 1.506	0.204			
	MCF	1.176	0.870 – 1.589	0.291			
	PVI 5FU + MMC	2.107	1.461 – 3.040	<0.001			
	PVI 5FU	2.132	1.481 – 3.067	<0.001			
	Overall			<0.001			
Study	Trial 3 ¹⁰ (r)	1.000			1.000		
	Trial 1 ¹⁵	0.993	0.804 – 1.228	0.951	1.034	0.830 – 1.288	0.763
	Trial 2 ¹⁶	1.850	1.432 – 2.390	<0.001	1.736	1.326 – 2.271	<0.001
	Overall			<0.001			<0.001

All variables with a p-value of less than 0.2 in univariate analyses were entered into a multivariate model in a forward stepwise manner;
r=reference, mets=metastases

Table 3: Objective response rates and toxicity composite endpoint

	Adeno	SCC
Number of patients	841	132
Complete response	48 (6%)	7 (5%)
Partial Response	323 (38%)	36 (27%)
Stable disease	224 (30%)	35 (27%)
Progressive disease	157 (19%)	38 (29%)
Objective response rate (95% CI)	371 (44%) 41-48%	43 (33%) 25-41% p=0.01
Toxicity composite endpoint (95% CI)	381 (45%) 42 – 49%	58 (44%) 35 – 53% p=0.77

Figure 1: Analysis profile



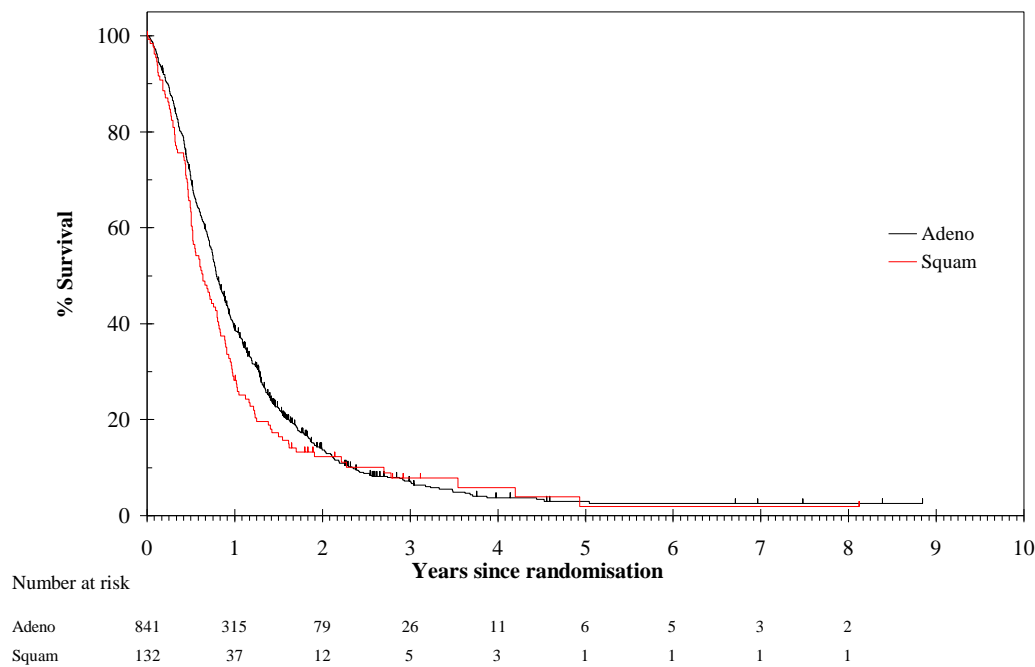
* Includes carcinoma, undifferentiated carcinoma, adenosquamous carcinoma

Figure 1 Legend

CONSORT diagram indicating the derivation of eligible patients in this analysis.

Figure 2: Overall survival

A According to histology (all patients)



B According to histology (excluding OGJ tumours)

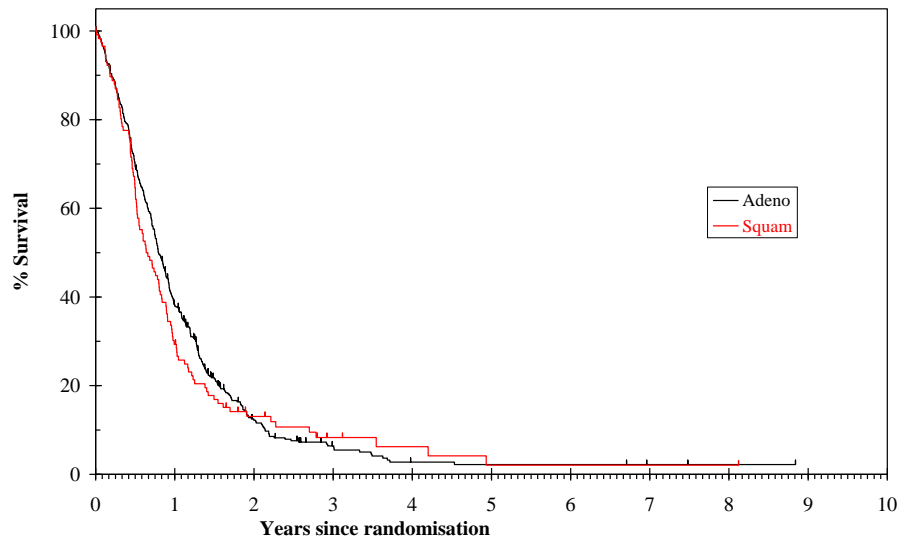


Figure 2 Legend

A Overall survival according to histology (adenocarcinoma=841 patients, SCC = 132 patients). The HR for death in the adenocarcinoma group compared to the SCC group was 0.85 (95% CI 0.70-1.03, p=0.09)

B Overall survival according to histology excluding OGJ tumours (adenocarcinoma= 438 patients, SCC= 117 patients). The HR for death for the adenocarcinoma group compared to the SCC group was 0.91 (95% CI 0.73-1.13, p=0.38)

Figure 3: Time to developing toxicity composite endpoint (TCE) according histology

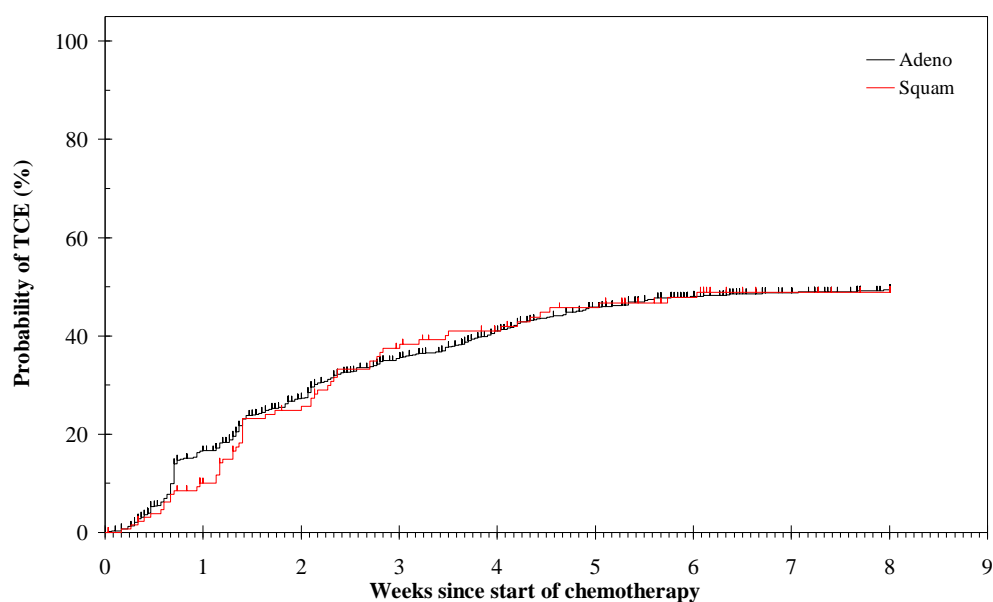


Figure 3 Legend

The time to development of the Toxicity Composite Endpoint (TCE) is shown for patients with adenocarcinoma (n=841) versus SCC (n=132).

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